



THE UNIVERSITY *of* EDINBURGH

Edinburgh Research Explorer

Pathogenesis of chronic obstructive pulmonary disease

Citation for published version:

Macnee, W 2005, 'Pathogenesis of chronic obstructive pulmonary disease', *Proceedings of the American Thoracic Society*, vol. 2, no. 4, discussion 290-1, pp. 258-66 . <https://doi.org/10.1513/pats.200504-045SR>

Digital Object Identifier (DOI):

[10.1513/pats.200504-045SR](https://doi.org/10.1513/pats.200504-045SR)

Link:

[Link to publication record in Edinburgh Research Explorer](#)

Document Version:

Publisher's PDF, also known as Version of record

Published In:

Proceedings of the American Thoracic Society

General rights

Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy

The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.



Pathogenesis of Chronic Obstructive Pulmonary Disease

William MacNee

Edinburgh Lung and the Environment Group Initiative/Colt Research Laboratories, Medical School, University of Edinburgh, Edinburgh, Scotland, United Kingdom

The current paradigm for the pathogenesis of chronic obstructive pulmonary disease is that chronic airflow limitation results from an abnormal inflammatory response to inhaled particles and gases in the lung. Airspace inflammation appears to be different in susceptible smokers and involves a predominance of CD8⁺ T lymphocytes, neutrophils, and macrophages. Studies have characterized inflammation in the peripheral airspaces in different stages of disease severity. Two other processes have received considerable research attention. The first is a protease–antiprotease imbalance, which has been linked to the pathogenesis of emphysema. However, the hypothesis of an increased protease burden associated with functional inhibition of antiproteases has been difficult to prove and is now considered an oversimplification. The second process, oxidative stress, has a role in many of the pathogenic processes of chronic obstructive pulmonary disease and may be one mechanism that enhances the inflammatory response. In addition, it has been proposed that the development of emphysema may involve alveolar cell loss through apoptosis. This mechanism may involve the vascular endothelial growth factor pathway and oxidative stress.

Keywords: apoptosis; emphysema; inflammation; oxidative stress; protease–antiprotease imbalance

Chronic obstructive pulmonary disease (COPD) is a slowly progressive condition characterized by airflow limitation, which is largely irreversible (1). Cigarette smoking is the main etiologic factor in this condition, far outweighing any of the other risk factors. The pathogenesis of COPD is therefore strongly linked to the effects of cigarette smoke on the lungs. There is a general relationship between the extent of the smoking history and the severity of the airflow limitation; however, there is a huge individual variation. Fletcher and Peto (2), in an 8-yr prospective study of working men in West London, showed that the average decline in FEV₁ in smokers is faster (60 ml/yr) than in nonsmokers (30 ml/yr). However, smokers who develop COPD have an average decline in FEV₁ of greater than 60 ml/yr, and only 15 to 20% of smokers develop clinically significant COPD. It is from these studies that the concept of the susceptible smoker developed.

SUBTYPES OF COPD

Chronic Bronchitis

The cough and sputum production that define chronic bronchitis result from an innate immune response to inhaled toxic particles and gases in cigarette smoke. In chronic bronchitis there is inflammation in the epithelium of the central airways and in the mucus-producing glands (3). This airway inflammation is associ-

ated with increased mucus production, reduced mucociliary clearance, and increased permeability of the airspace epithelial barrier.

The contribution of mucus hypersecretion to the airflow limitation in COPD is still uncertain. It appears that it contributes little in the early stages of COPD, because mucus production in smokers with normal lung function does not appear to predict later development of COPD (4). However, chronic mucus hypersecretion may contribute in the later stages of the disease, because of an increased risk of exacerbations that may accelerate the loss of FEV₁. Chronic mucus hypersecretion may be a reflection of the inflammatory response in the submucosal glands (2). Inflammatory cells release serine proteases that are potent secretagogues for mucus (5). Oxidants derived from cigarette smoke and released from inflammatory leukocytes may also be involved in overproduction of mucin by induction of the *MUC5AC* gene (6).

Emphysema

Emphysema is defined as enlargement of the distal airspaces, beyond the terminal bronchioles, caused by destruction of the airway walls (7). Emphysematous lung destruction reduces maximal expiratory airflow by decreasing the elastic recoil force that drives air out of the lungs. The centrilobular or centriacinar form of emphysema results from dilation or destruction of the respiratory bronchioles and is the type of emphysema most closely associated with tobacco smoking. The panlobular or panacinar form of emphysema, which is usually associated with α_1 -antitrypsin (α_1 -AT) deficiency, results in more even dilation and destruction of the entire acinus. It has been suggested that one or the other of these types predominates in severe disease and that the centriacinar type is associated more with severe small-airway obstruction (8).

There is a relationship between the degree of emphysema and pack-yr of smoking, but not a strong one. Only about 40% of heavy smokers develop substantial lung destruction from emphysema, and emphysema can be found in some individuals who have normal lung function (3).

Small-Airway Disease

A major site of airway obstruction in COPD is the smaller conducting airways (< 2 mm in diameter) (9). Studies have shown that there are structural abnormalities in small airways in smokers with and without COPD (10). There is also a relationship between the severity of COPD and the extent of occlusion of the airway lumen by inflammatory mucous exudates. Inflammation and peribronchial fibrosis contribute to the fixed airway obstruction in the small airways in COPD, and progression of the inflammation, resulting in destruction of the alveolar attachments on the outer walls of the small airways, may also contribute.

INFLAMMATION IN THE LUNGS IN COPD

Studies of lung or bronchial biopsies and induced sputum have shown evidence of lung inflammation in all cigarette smokers. However, it appears that an enhanced or abnormal inflammatory response to inhaled particles or gases, beyond the normal protec-

(Received in original form April 22, 2005; accepted in final form June 1, 2005)

Supported by National Institutes of Health grant 1 ROI HL72282-01.

Correspondence and requests for reprints should be addressed to W. MacNee, M.D., Respiratory Medicine, ELEG/Colt Research Laboratories, Wilkie Building, Medical School, Teviot Place, Edinburgh EH8 9AG, UK. E-mail: w.macnee@ed.ac.uk

Proc Am Thorac Soc Vol 2, pp 258–266, 2005

DOI: 10.1513/pats.200504-045SR

Internet address: www.atsjournals.org

TABLE 1. VARIATION OF INFLAMMATORY CELLS AND MARKERS OF INFLAMMATION IN THE BRONCHIAL SUBMUCOSA

	CD45	CD3	Neutrophils	EOS	Mast Cells	CD68	CD8	CD4
Severe COPD	—	↓ 23	↑ 14	→ 14	→ 14	↑ 14	↓ 23	→ 23
Mild/moderate COPD	↑ 99, 100	↑ 13, 14, 99, 101 → 23	→ 99, 100 ↑ 14	→ 14, 99, 100	→ 14, 99, 100	↑ 99, 100 → 14	↑ 13, 100 → 23, 99	→ 13, 23, 99, 100
Control smokers	→ 100	→ 23, 100 ↑ 13	→ 14, 100	→ 14, 100	→ 14, 100	→ 14, 100	↑ 13 → 23, 100	→ 13, 23, 100
Control nonsmokers	→ 99, 100	→ 13, 99, 100, 101	→ 99, 100	→ 99, 100	→ 99, 100	→ 99, 100	→ 13, 99, 100	→ 13, 99, 100

Definition of abbreviations: COPD = chronic obstructive pulmonary disease; EOS = eosinophils.

↑ = significantly increased values in comparison with that indicated by →; → = basal values or values nonsignificantly changed; ↓ = significantly decreased values in comparison with that indicated by →. Numbers close to the arrows indicate references.

Adapted from Di Stefano and coworkers (12).

tive inflammatory response in the lungs, is a characteristic feature of COPD and has the potential to produce lung injury (11). Both innate and adaptive inflammatory and immune responses are involved in the lung inflammation in smokers and in patients with COPD. Studies have begun to characterize the lung inflammation in COPD in terms of its type, site, and degree, and the relationship to severity of disease.

Studies of bronchial biopsy specimens from patients with mild to moderate COPD show an increase in inflammatory cell infiltration in the central airways, compared with nonsmokers or smokers who have not developed the disease (12). In the bronchial mucosa in patients with COPD, T lymphocytes predominate, mainly CD8⁺ cells and macrophages (CD68⁺ cells; Table 1). It has been suggested that the presence of increased CD8⁺ T lymphocytes differentiates between smokers who do and do not develop COPD and that there is a correlation between T-cell numbers, the amount of alveolar destruction, and the severity of airflow limitation. However, smokers with normal lung function also show, to a lesser extent, an increased number of CD8⁺ cells compared with control nonsmokers (13). Indeed, there is a decrease in T-lymphocyte infiltration in bronchial biopsy specimens from subjects with severe COPD (14).

The mechanism by which CD8⁺ T lymphocytes accumulate in the airways of the lungs in COPD is not fully understood. T cells in peripheral airways in patients with COPD show increased expression of CXCR3, a receptor activated by interferon-inducible protein 10, and expression of interferon-inducible protein 10 itself is increased in bronchiolar epithelial cells. This could contribute to the accumulation of CD8⁺ cells, which preferentially express CXCR3. Circulating CD8⁺ cells are also increased in number in patients with COPD who do not smoke (15), and there is an increase in CD4⁺ cells in patients with COPD, particularly as the disease progresses (16, 17). This suggests chronic immune stimulation. It may be that chronic colonization of the lower respiratory tract of patients with COPD by bacterial and viral pathogens is responsible for this enhanced inflammatory response (10). Studies suggest an increase in B lymphocytes and in bronchial-associated lymphoid tissue in small airways as the disease progresses (10). It is also possible that cigarette smoke itself damages airway cells, creating new autoantigens that drive the immunoinflammatory response (18).

The role of T cells in the pathogenesis of COPD is not fully understood. CD8⁺ cells have the potential to release tumor necrosis factor α , perforins, and granzymes, in addition to activating the Fas–Fas ligand apoptotic pathway. An association has been shown between CD8⁺ cells and apoptosis of alveolar epithelial cells in subjects with emphysema (19).

Increased numbers of activated neutrophils are found in sputum from patients with COPD (20). The lack of significant increased neutrophil numbers in the lung parenchyma may be due to the fact that these cells make a rapid transit through the airways and

the lung parenchyma (21). Neutrophils have the potential to secrete serum proteinases, including neutrophil elastase, cathepsin G, and proteinase 3, as well as matrix metalloproteinase 8 (MMP-8) and MMP-9. These proteases may contribute to alveolar destruction and are also potent stimuli of mucus secretion.

The role that neutrophils play in the pathogenesis of COPD is not entirely clear. Relationships have been shown between circulating neutrophils and the fall in FEV₁ (22). Similarly, neutrophil numbers in bronchial biopsy specimens and induced sputum are related to disease severity (14) and the rate of decline in lung function (23).

Cigarette smoking is known to increase circulating neutrophil leukocyte count and to cause sequestration of neutrophils in the lung capillaries (24) by decreasing their deformability. Cigarette smoke also has a direct stimulatory effect on granulocyte production in the bone marrow, possibly mediated by granulocyte-macrophage colony-stimulating factor and granulocyte colony-stimulating factor released from macrophages (25). It is possible that neutrophils are activated within the pulmonary microcirculation to release reactive oxidant species and proteases that may have a direct injurious effect.

Once sequestered, neutrophils adhere to endothelial cells, and the adhesion molecule E-selectin has been shown to be upregulated in the airway epithelial cells of patients with COPD (26). Neutrophils can then migrate to the respiratory tract under the control of chemotactic factors, such as leukotriene B₄, interleukin 8 (IL-8), and related CXC chemokines, including growth-related oncogene- α and epithelial cell–derived neutrophil attractant 78. These chemotactic factors have been shown to be increased in the airways in patients with COPD (27, 28).

There is a 5- to 10-fold increase in the numbers of macrophages in the airways, lung parenchyma, and bronchoalveolar lavage fluid (BALF) in patients with COPD. Macrophage numbers in the airways correlate with the severity of COPD (29). Cigarette smoke activates macrophages to release inflammatory mediators, including tumor necrosis factor α , IL-8 and other CXC chemokines, monocyte chemotactic peptide-1, leukotriene B₄, and reactive oxygen species. Macrophages also secrete proteases, including MMP-2, MMP-9, and MMP-12; cathepsins K, L, and S; and neutrophil elastase, taken up from neutrophils. Compared with macrophages from normal smokers, those from patients with COPD are more activated, secrete more inflammatory proteins, and have greater elastolytic activity, which is further enhanced by exposure to cigarette smoke (30, 31). Increased numbers of macrophages in the lungs of patients with COPD and in the lungs of smokers may result from increased recruitment of monocytes from the circulation in response to monocyte chemotactic chemokines such as monocyte chemotactic peptide-1, which has been shown to be increased in sputum and BALF in patients with COPD (31). CXC chemokines also act as chemoattractants to monocytes. The concentration of growth-related

TABLE 2. VARIATION OF INFLAMMATORY CELLS AND MARKERS OF INFLAMMATION IN THE CENTRAL AND PERIPHERAL AIRWAYS

	Central Airways						Peripheral Airways (< 3 mm in diameter)			
	Neutrophils	CD68	CD4	CD8	IL-4	IL-5	Neutrophils	CD68	CD4	CD8
Mild/moderate COPD	→ 102	→ 102	→ 102	→ 102	↓ 103	→ 103	→ 36, 104–106	↑ 106, 107 → 36, 104, 105	→ 36, 104–106	↑ 36 → 104–107
Chronic bronchitis with normal FEV ₁	—	—	—	—	↑ 103	→ 103	—	—	—	—
Control smokers	→ 102	→ 102	→ 102	→ 102	→ 103	→ 103	→ 36, 104–106	→ 36, 104–107	→ 36, 104–107	→ 36, 104–107

Definition of abbreviations: COPD = chronic obstructive pulmonary disease; IL = interleukin.

↑ = significantly increased values in comparison with that indicated by →; → = basal values or values nonsignificantly changed; ↓ = significantly decreased values in comparison with that indicated by →. Numbers close to the arrows indicate references.

Adapted from Di Stefano and coworkers (12).

oncogene- α is increased markedly in sputum and BALF from patients with COPD. Furthermore, monocytes from patients with COPD show a greater chemotactic response to growth-related oncogene- α than do cells from normal smokers and non-smokers (32).

There is an increased number of dendritic cells in the airways and alveolar walls of smokers (33). The role of dendritic cells in COPD is not yet defined, but they may have an important function in the innate and adaptive immune responses in COPD.

Airway epithelial cells can be activated by cigarette smoke to produce inflammatory mediators, including tumor necrosis factor α , IL-1 β , granulocyte-macrophage colony-stimulating factor, and IL-8. The epithelium in the small airways may be an important source of transforming growth factor β (TGF- β), adding to the induction of local fibrosis (34). Epithelial cells can also secrete antioxidants, secrete antiproteases, and transport immunoglobulin- α , so they may be involved in adaptive immunity. Cigarette smoke may impair these innate and adaptive immune responses of the airway epithelium and increase the likelihood of infection.

Many of the inflammatory mediators that are expressed in COPD are controlled by the transcription factor nuclear factor (NF)- κ B, which is upregulated in alveolar macrophages in patients with COPD and in airway cells in patients with mild/moderate COPD in comparison with control nonsmokers (35). Upregulation of NF- κ B in lung cells in COPD may be a key molecular mechanism involved in the ongoing inflammatory process in the airways.

In general, with increasing severity of COPD there is a further enhancement of the inflammatory response. Compared with mild/moderate disease, there is a further increase in expression of inflammatory proteins such as macrophage inflammatory protein 1 α , a chemokine involved in the activation of mononuclear cells and granulocytes. There are also further increases in the number of neutrophils and macrophages in severe disease and a decrease

in T lymphocytes (CD3⁺ cells). There appears to be a shift in the cellular type in severe disease toward cells with a phagocytic and proteolytic role in the bronchial tissues (Tables 1–3).

A comparison of the central and peripheral airways shows an increase in the total inflammatory cells in the peripheral airways (> 3 mm in diameter) in patients with chronic bronchitis with normal lung function, compared with control smokers (Table 2). Some studies have shown an increase in total inflammation and an increase in CD8⁺ cells in the peripheral airways of patients with mild/moderate COPD in comparison with control smokers (36).

The inflammatory response in the peripheral airways may play a role in the fibrosis that characterizes the small airways in patients with moderate/severe COPD. Studies that have assessed tissue obtained from lung volume reduction surgery in patients with severe COPD have shown an increase in total leukocytes and in CD4⁺ and CD8⁺ lymphocytes in both the peripheral airways and the lung parenchyma (12). In contrast, smokers with normal lung function show an increased number of macrophages and T lymphocytes in lung parenchyma compared with control nonsmokers, with no changes in CD4⁺ and CD8⁺ cells. In patients with mild to moderate COPD there is an increase in CD8⁺ cells in the alveolar septae compared with control nonsmokers (37, 38), and there is no change in the numbers of neutrophils, macrophages, or CD4⁺ cells.

PROTEASES AND ANTIPROTEASES

A large body of literature has been amassed to test the hypothesis that a protease–antiprotease imbalance, leading to the breakdown of connective tissue components, particularly elastin, is the critical mechanism in the pathogenesis of emphysema in smokers. This concept developed from studies of early-onset emphysema in α_1 -AT–deficient patients. Elastin is an important target for

TABLE 3. VARIATION OF INFLAMMATORY CELLS IN THE LUNG PARENCHYMA

	Neutrophils	CD68	CD3	CD4	CD8	Eosinophils
Mild/moderate COPD	→ 38, 92	→ 38	↑ 92	→ 38, 92	↑ 38, 92	→ 38
Smokers with normal FEV ₁	→ 37, 38, 92	↑ 37	↑ 37	→ 38, 92	→ 38, 92	→ 38
		→ 38	→ 92			
Control nonsmokers	↑ 37	→ 37, 38	→ 37, 92	→ 38, 92	→ 38, 92	→ 38
	→ 38, 92					

Definition of abbreviation: COPD = chronic obstructive pulmonary disease.

↑ = significantly increased values in comparison with that indicated by →; → = basal values or values nonsignificantly changed; ↓ = significantly decreased values in comparison with that indicated by →. Numbers close to the arrows indicate references.

Adapted from Di Stefano and coworkers (12).

proteolytic enzymes, and its destruction results in loss of elasticity in the lung parenchyma.

Elastin is the principal component of elastic fibers and is secreted from several cell types as a precursor, tropoelastin. These tropoelastin molecules become aligned in the extracellular space on microfibrils. Under the action of lysyl oxidase, the lysine residues in tropoelastin are modified, which causes the tropoelastin monomers to cross-link and form larger, insoluble elastin polymers. Because the cross-links, known as desmosines, are unique to elastin, they have been used as a marker of elastin degradation (39). Desmosine and elastin peptides are elevated in smokers and patients with COPD. However, there has been controversy over the specificity of measurements of these peptides in urine, particularly as a reflection of lung elastin degradation alone, because of the extreme durability of lung elastin. There is minimal elastin turnover in normal subjects, so breakdown products should not be detectable. Nevertheless, studies indicate that the annual rate of decline in FEV₁ in a group of smokers correlated positively with urine levels of desmosine (40). The validity of the use of desmosine or elastin peptides as a marker of elastolysis remains unresolved.

Together with destruction of elastin, inactivation of antiproteases is central to the protease-antiprotease imbalance hypothesis. Early studies showed that the function of α_1 -AT was reduced by about 40% in smokers, compared with nonsmokers (41). This "functional α_1 -AT deficiency" was believed to be due to inactivation of α_1 -AT by oxidants in cigarette smoke. However, most of the α_1 -AT in cigarette smokers remains active and is therefore still capable of protecting against the increased protease burden. There is only a transient and nonsignificant fall in α_1 -AT activity in BALF 1 h after smoking (42). Thus, studies assessing the function of α_1 -AT in either chronic or acute cigarette smoking have not been definitive. It is clear, however, that the hypothesis that the major event is an imbalance between an increased elastase burden in the lungs and a "functional deficiency" of α_1 -AT, because of its inactivation, is an oversimplification.

As discussed above, there is substantial evidence that the numbers of neutrophils and macrophages are increased in the airspaces in chronic smokers. The elastase burden could be increased in cigarette smokers by enhanced degranulation and therefore release of elastase. There is some evidence to support this, because neutrophils isolated from patients with emphysema show greater elastase-induced fibronectin degradation *in vitro* than do cells from control subjects matched for age and smoking history (43).

Further hypotheses have invoked a contributory role for other antiproteases, such as antileukoprotease, or more subtle changes, for example, a decrease in the association rate constant of α_1 -AT for neutrophil elastase, which may contribute to elastin degradation.

Elastin Synthesis and Repair

There is some evidence supporting the concept that an abnormality in elastin synthesis and repair may be involved in the pathogenesis of emphysema. In animal models of intratracheal instillation of elastases, lung elastin is depleted within hours to a few days (44), followed by increased elastin synthesis over a period of weeks. However, in areas of emphysema in these models, alveolar elastic fibers have an abnormal appearance (45) and resemble the aberrant elastic fibers in human emphysema. Thus, although elastin synthesis after injury restores the elastin content of the lungs, it does not restore normal lung architecture in these experimental models.

In an animal model of elastase-induced emphysema, treatment with retinoic acid restored normal alveolar architecture.

These studies in adult male rats (which have continued lung growth throughout their adult life, in contrast with humans) must be verified, but they provide some intriguing evidence that the destructive process in emphysema, which was always considered irreversible, may be capable of repair (46).

In addition to serum proteases, cysteine proteases (cathepsins) may have a role in COPD. Cathepsin C was induced in mice by overexpression of IFN- γ , which induces emphysema (47). Cathepsin inhibitors have been shown to reduce emphysema induced by overexpression of IL-13 in mouse lung (48). Cathepsin L has been detected in BALF from patients with emphysema (49), and alveolar macrophages in patients with COPD secrete more cysteine proteases than do macrophages from normal smokers or nonsmokers (50).

There is increasing interest in the role of MMPs in COPD (51). Increased concentrations of MMP-1 (collagenase) and MMP-9 (gelatinase B) are present in BALF from patients with COPD (52, 53), and there is increased activity of MMP-9 in the lung parenchyma of patients with emphysema (54, 55). MMP-1 expression is also increased in the lungs of patients with emphysema, particularly in type II pneumocytes (56). Alveolar macrophages from smokers express more MMP-9 than do those from normal subjects (30), and there is an even greater increase in patients with COPD (29). Animal models have shown that cigarette smoke-induced emphysema does not occur in mice lacking MMP-12. In such mice, emphysema induced by IL-13 or IFN- γ expression is also reduced (48), associated with a marked reduction in monocyte recruitment to the lungs. MMPs are also known to activate the latent form of TGF- β to its active form. In mice lacking the integrin $\alpha_v\beta_6$, there is a failure to activate TGF- β , and the animals do not develop age-related emphysema, which can be prevented by overexpression of TGF- β_1 (57). These data suggest that TGF- β_1 may downregulate MMP-12 under normal conditions and that the absence of TGF- β results in excessive MMP-12 production and emphysema.

Mice lacking MMP-9 are not protected against emphysema caused by cigarette smoke but are protected from small-airway fibrosis (58). TGF- β is activated by MMP-9, and this mechanism could provide a link between enhanced elastolytic activity by MMP-9 and the simultaneous production of fibrosis by activation of TGF- β . It appears that, although MMP-12 is an important protease in the mouse, it is not as important in humans as MMP-9. Macrophages from patients with COPD have a blunted response to stimuli for the release of tissue inhibitor of metalloproteinase 1, which would favor increased elastolysis (29).

ROLE OF OXIDANTS AND ANTIOXIDANTS IN SMOKING-INDUCED COPD

Cigarette smoke is a complex mixture of more than 4,700 chemical compounds, including high concentrations of free radicals and other oxidants. Other sources of reactive oxygen species are those generated through normal cellular processes in the lungs, such as those produced by normal cellular respiration or by inhalation of air pollutants such as particulate pollution.

A delicate balance exists between the toxicity of oxidants and the protective effects of intra- and extracellular antioxidant defense systems, which are critically important for the maintenance of normal pulmonary cellular functions. A shift of the oxidant/antioxidant balance in favor of oxidants is known as oxidative stress. There is now considerable evidence of increased oxidative stress in smokers and in patients with COPD (59).

Cigarette smoke contains free radicals in both the gas and tar phases (60). Short-lived radicals in the gas phase of cigarette smoke may be quenched immediately in the lung epithelial lining

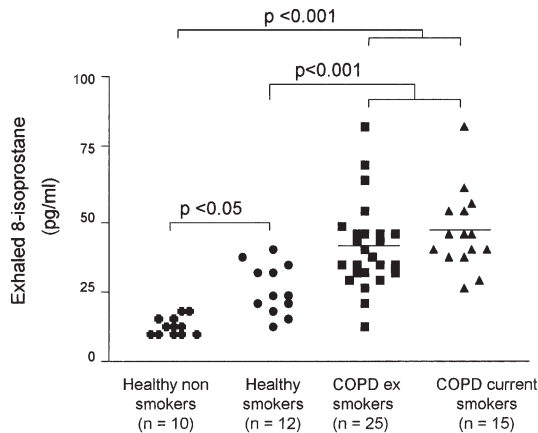


Figure 1. Concentrations of 8-isoprostane, a lipid peroxidation product, in exhaled breath condensate. Mean values are shown by horizontal bars. COPD = chronic obstructive pulmonary disease. Modified by permission from Reference 71.

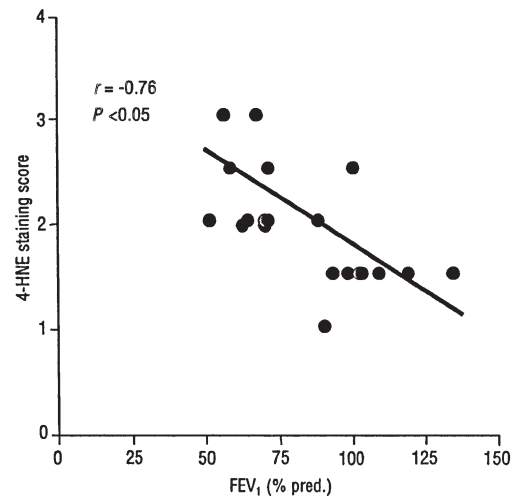


Figure 2. Correlation between lung function and levels of 4-hydroxy-2-nonenal (4-HNE), a lipid peroxidation product, in the lungs. Modified by permission from Reference 72.

fluid; however, redox reactions in cigarette smoke condensate may produce reactive oxygen species for a considerable time.

The oxidant burden in lungs may be further enhanced in smokers by the increased numbers of neutrophils and macrophages in the alveolar space. *In vitro* studies have shown that alveolar leukocytes from cigarette smokers spontaneously release increased amounts of oxidants, such as $O_2^{\cdot -}$ and H_2O_2 , compared with those from nonsmokers (61).

Components of the lung matrix (e.g., elastin and collagen) can be directly damaged by oxidants in cigarette smoke (62). Furthermore, cigarette smoke can interfere with elastin synthesis and repair (63), potentially leading to the development of emphysema.

All tissues are vulnerable to oxidant damage, but by virtue of its direct contact with the environment, the airspace epithelial surface of the lung is particularly vulnerable. Injury to the epithelium, manifested as an increase in airspace epithelial permeability, may be an important early event after exposure to cigarette smoke (64). Extra- and intracellular glutathione, an antioxidant, appears to be critical to the maintenance of epithelial integrity after exposure to cigarette smoke. This was shown in studies in which the increased permeability of epithelial cell monolayers *in vitro*, and in rat lungs *in vivo*, after exposure to cigarette smoke condensate, was associated with profound changes in the homeostasis of glutathione (65, 66).

These *in vitro* and animal studies are paralleled by human studies demonstrating increased epithelial permeability in chronic smokers compared with nonsmokers, with a further increase in epithelial permeability after acute smoking (67). Thus, cigarette smoke has a detrimental effect on alveolar epithelial cell function that is, in part, oxidant mediated.

A major site of free radical attack is on polyunsaturated fatty acids in cell membranes producing lipid peroxidation, a process that may continue as a chain reaction to generate hydroperoxides and long-lived aldehydes. Levels of products of lipid peroxidation in plasma and BALF are significantly increased in healthy smokers and patients with acute exacerbations of COPD, compared with healthy nonsmokers (64, 67, 68).

Several studies demonstrate increased levels of oxidants in exhaled air or breath condensates (69), although measurements of exhaled breath condensate have inherent problems and standardization of this technique has still to be accomplished. There is an increased concentration of H_2O_2 in exhaled breath condensate in patients with COPD (70). Concentrations of a lipid peroxidation product in exhaled breath condensate are increased even in patients who are ex-smokers (Figure 1) (71). Furthermore,

there is evidence that oxidative stress can cause increased lipid peroxidation in lung tissue in patients with COPD compared with smokers who have a similar smoking history but have not developed the disease (72). In that study, the level of lipid peroxidation correlated with the degree of airflow limitation (Figure 2).

Free iron is a critical element in many oxidative processes. Macrophages from smokers have been shown to contain more iron than those from nonsmokers, and they release more iron, thus potentially increasing the oxidant burden in smokers (73).

The major antioxidants in respiratory tract lining fluid include mucin, reduced glutathione, uric acid, protein (largely albumin), and ascorbic acid (74). There is limited information about respiratory epithelial antioxidant defenses in smokers, and even less about those in patients with COPD. Studies have shown that glutathione is elevated in BALF from chronic smokers (64, 75). Even so, glutathione may not be present in sufficient quantities to deal with the excessive oxidant burden during acute smoking, as cigarette smoke exposure depletes glutathione in a dose- and time-dependent manner (76).

Reduced levels of vitamin E are present in the BALF of smokers compared with nonsmokers (77). By contrast, other studies found a marginal increase in vitamin C in the BALF of smokers, compared with nonsmokers (78). The apparent discrepancy may be due to different smoking histories in chronic smokers, in particular the time of smoking the last cigarette in relation to the sampling of BALF.

Expression of glutathione peroxidase and superoxide dismutase, another antioxidant enzyme, is elevated in the lungs of rats exposed to cigarette smoke (79, 80).

OTHER MECHANISMS RELATED TO THE PATHOGENESIS OF COPD

Mechanisms Related to Inflammation

Studies have suggested that susceptibility to COPD may be related to latent adenoviral infection. These studies have demonstrated the ability of adenoviral E1A proteins, which associate with DNA, to enhance the binding of a number of transcription factors to their nuclear consensus sites and so activate a wide variety of genes (81). The E1A protein occurs more commonly in the lungs of smokers with COPD than in smokers who have not developed the disease (82). Furthermore, it has been shown

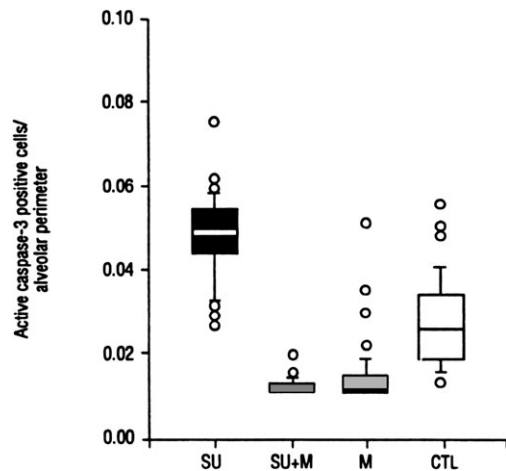


Figure 3. Rat lungs treated with SU5416 (SU), a vascular endothelial growth factor receptor blocker, show increased alveolar cell apoptosis, as quantified by an active caspase-3 assay. M40419 (M), a superoxide dismutase mimetic, has a protective effect on SU5416-induced apoptosis. CTL = control. Modified by permission from Reference 97.

in an animal model that latent adenoviral infection increases the inflammation that follows exposure to cigarette smoke (83). Transfection of an E1A-type human epithelial cell line results in increased activation of NF- κ B, and consequently increased release of IL-8 in response to cell activation and increased production of TGF- β , suggesting a molecular mechanism for the amplification of inflammatory response (84, 85).

A further mechanism that may amplify inflammation in COPD may be imbalance between histone acetylation and deacetylation, resulting in chromatin remodeling to a configuration that enhances inflammatory gene expression. Macrophages of cigarette smokers show a decrease in histone deacetylase activity (86), as has also been shown in the lungs of smoke-exposed animals (87). Decreased histone deacetylation enhances DNA unwinding and hence increases transcription of inflammatory genes. Preliminary evidence also suggests that histone deacetylase protein and activity are reduced in lung tissue in COPD, which may be the mechanism that enhances lung inflammation (88, 89).

There is evidence that smoking cessation does not resolve the inflammatory response in the airways, particularly in advanced COPD. Molecular mechanisms such as transcription factor activation and chromatin remodeling, perhaps as a result of increased oxidative stress, might be responsible for perpetuating the inflammatory process.

Gene expression profiling (90, 91) of human lung tissue from smokers and smokers with severe emphysema suggests an increase in transcripts encoding proteins involving inflammation immune responses and proteolysis, and differences in gene profiling have been found between patients with emphysema that is induced by cigarette smoking and patients with emphysema that is related to α_1 -antitrypsin. Such studies of gene profiling in human lung tissue should provide insight into the pathogenesis and may allow distinction between different phenotypes of disease and identify targets for therapeutic intervention.

Apoptosis and Emphysema

As explained previously, the traditional paradigm for alveolar wall destruction in emphysema has been that increased inflammatory response results in a protease–antiprotease imbalance. Now, studies have demonstrated apoptosis in human emphyse-

matous lungs (92, 93). A hypothesis has been advanced that the alveolar cell loss in emphysema is due to apoptosis in response to cigarette smoke, mediated by blockade of the vascular endothelial growth factor (VEGF) receptor that occurs in emphysematous lungs. Indeed, rats in which VEGF receptors have been blocked develop emphysema (94). Decreased levels of VEGF in induced sputum have been shown to correlate with the degree of airflow limitation and alveolar destruction in patients with emphysema (95). Furthermore, mice develop emphysema after a single intratracheal injection of active caspase-3, an inducer of apoptosis, plus a protein transfection agent (96). Another study has shown that oxidative stress and apoptosis interact in rats and cause emphysema due to VEGF receptor blockade (Figure 3) (97).

One study (98) has also shown that apoptosis in lung tissue was correlated inversely with surface area and that emphysematous lungs demonstrated decreased surface area and increased cell proliferation. However, there was no correlation between apoptosis and proliferation, which suggests that although both proliferation and apoptosis increase in emphysema they are not in equilibrium, which potentially would contribute to a reduction in lung surface area.

CONCLUSION

No single mechanism can account for the complex pathology in COPD. It is likely that interactions occur between different mechanisms. For example, there are probably interrelationships between the protease–antiprotease balance, oxidative stress, and apoptosis as destructive processes in emphysema. Better understanding of the relative importance of these different pathogenic mechanisms will come from proof-of-concept therapeutic intervention studies.

Conflict of Interest Statement: W.M. has been reimbursed for travel by GlaxoSmithKline, Zambon, AstraZeneca, Boehringer Ingelheim, Pfizer, and Micromet for attending conferences. He has received honoraria from GlaxoSmithKline, AstraZeneca, Zambon, and Pfizer for participating as a speaker in scientific meetings. He serves on advisory boards for GlaxoSmithKline, Pfizer, Almirall, Amgen, Bayer, and Micromet. He serves as a consultant for Pfizer and SMB Pharmaceuticals. Research grants to support work carried out in his laboratory come from SMB, Pfizer, Ceremedix, GlaxoSmithKline, Chugi, and Novartis.

References

1. Celli BR, MacNee W; ATS/ERS Task Force. Standards for the diagnosis and treatment of patients with COPD: a summary of the ATS/ERS position paper. *Eur Respir J* 2004;23:932–946.
2. Fletcher C, Peto R. The natural history of chronic airflow obstruction. *BMJ* 1977;1:1645–1658.
3. Hogg JC. Pathophysiology of airflow limitation in chronic obstructive pulmonary disease. *Lancet* 2004;364:709–721.
4. Vestbo J, Lange P. Can GOLD stage 0 provide information of prognostic value in chronic obstructive pulmonary disease? *Am J Respir Crit Care Med* 2002;166:329–332.
5. Sommerhoff CP, Nadel JA, Basbaum CB, Caughey GH. Neutrophil elastase and cathepsin G stimulate secretion from cultured bovine airway gland serous cells. *J Clin Invest* 1990;85:682–689.
6. Shao MXG, Nakanaga T, Nadel JA. Cigarette smoke induces MUC5AC mucin overproduction via tumor necrosis factor- α -converting enzyme in human airway epithelial (NCI-H292) cells. *Am J Physiol Lung Cell Mol Physiol* 2004;287:L420–L427.
7. Snider GL, Kleinerman LJ, Thurlbeck WM, Bengali ZH. The definition of emphysema: report of a National Heart, Lung, and Blood Institute, Division of Lung Diseases workshop. *Am Rev Respir Dis* 1985;132:182–185.
8. Kim WD, Eidelman DH, Izquierdo JL, Ghezzi H, Saetta MP, Cosio MG. Centrilobular and panlobular emphysema in smokers: two distinct morphologic and functional entities. *Am Rev Respir Dis* 1991;144:1385–1390.
9. Hogg JC, Macklem PT, Thurlbeck WM. Site and nature of airways obstruction in chronic obstructive lung disease. *N Engl J Med* 1968;278:1355–1360.

10. Hogg JC, Chu F, Utokaparch S, Woods R, Elliott WM, Buzatu L, Cherniack RM, Rogers RM, Sciurba FC, Coxson HO, Pare PD. The nature of small airway obstruction in chronic obstructive pulmonary disease. *N Engl J Med* 2004;350:2645–2653.
11. Sassetta M, Turato G, Luppi F. Inflammation in the pathogenesis of chronic obstructive pulmonary disease. In: Voelkel N, MacNee W, editors. Chronic obstructive lung disease. Hamilton, ON, Canada: BC Decker; 2002. pp. 114–126.
12. Di Stefano A, Caramori G, Ricciardolo FLM, Capelli A, Adcock IM, Donner CF. Cellular and molecular mechanisms in chronic obstructive pulmonary disease: an overview. *Clin Exp Allergy* 2004;34:1156–1167.
13. Di Stefano A, Caramori G, Oates T, Capelli A, Lusuardi M, Gnemmi I, Ioli F, Chung KF, Donner CF, Barnes PJ, et al. Increased expression of nuclear factor- κ B in bronchial biopsies from smokers and patients with COPD. *Eur Respir J* 2002;20:556–563.
14. Di Stefano A, Capelli A, Lusuardi M, Balbo P, Vecchio C, Maestrelli P, Mapp CE, Fabbri LM, Donner CF, Sassetta M. Severity of airflow limitation is associated with severity of airway inflammation in smokers. *Am J Respir Crit Care Med* 1998;158:1277–1285.
15. Kim WD, Kim WS, Koh Y, Lee SD, Lim CM, Kim DS, Cho YJ. Abnormal peripheral blood T-lymphocyte subsets in a subgroup of patients with COPD. *Chest* 2002;122:437–444.
16. Majori M, Corradi M, Caminati A, Cacciani G, Bertacco S, Pesci A. Predominant T_H1 cytokine pattern in peripheral blood from subjects with chronic obstructive pulmonary disease. *J Allergy Clin Immunol* 1999;103:458–462.
17. Retamales I, Elliott WM, Meshi B, Coxson HO, Pare PD, Sciurba FC, Rogers RM, Hayashi S, Hogg JC. Amplification of inflammation in emphysema and its association with latent adenoviral infection. *Am J Respir Crit Care Med* 2001;164:469–473.
18. Agusti A, MacNee W, Donaldson K, Cosio M. Hypothesis: does COPD have an autoimmune component? *Thorax* 2003;8:832–834.
19. Majo J, Ghezzi H, Cosio MG. Lymphocyte population and apoptosis in the lungs of smokers and their relation to emphysema. *Eur Respir J* 2001;17:946–953.
20. Keatings VM, Collins PD, Scott DM, Barnes PJ. Differences in interleukin-8 and tumor necrosis factor- α in induced sputum from patients with chronic obstructive pulmonary disease or asthma. *Am J Respir Crit Care Med* 1996;153:530–534.
21. Selby C, MacNee W. Factors affecting neutrophil transit during acute pulmonary inflammation: minireview. *Exp Lung Res* 1993;19:407–428.
22. Sparrow D, Glynn RF, Cohen M, Weiss ST. The relationship of the peripheral leukocyte count and cigarette smoking to pulmonary function among adult men. *Chest* 1984;86:383–386.
23. Stanescu D, Sanna A, Veriter C, Kostianev S, Calcagni PG, Fabbri LM, Maestrelli P. Airways obstruction, chronic expectoration and rapid decline in FEV₁ in smokers are associated with increased levels of sputum neutrophils. *Thorax* 1996;51:267–271.
24. MacNee W, Wiggs B, Belzberg AS, Hogg JC. The effect of cigarette smoking on neutrophil kinetics in human lungs. *N Engl J Med* 1989;321:924–928.
25. Terashima T, Wiggs B, English D, Hogg JC, van Eeden SF. Phagocytosis of small carbon particles (PM₁₀) by alveolar macrophages stimulates the release of polymorphonuclear leukocytes from bone marrow. *Am J Respir Crit Care Med* 1997;155:1441–1447.
26. Di Stefano A, Maestrelli P, Roggeri A, Turato G, Calabro S, Potena A, Mapp CE, Ciaccia A, Covacev L, Fabbri LM, et al. Upregulation of adhesion molecules in the bronchial mucosa of subjects with chronic obstructive bronchitis. *Am J Respir Crit Care Med* 1994;149:803–810.
27. Traves SL, Culpitt S, Russell REK, Barnes PJ, Donnelly LE. Elevated levels of the chemokines GRO- α and MCP-1 in sputum samples from COPD patients. *Thorax* 2002;57:590–595.
28. Tanino M, Betsuyaku T, Takeyabu K, Tanino Y, Yamaguchi E, Miyamoto K, Nishimura M. Increased levels of interleukin-8 in BAL fluid from smokers susceptible to pulmonary emphysema. *Thorax* 2002;57:405–411.
29. Russell RE, Culpitt SV, DeMatos C, Donnelly L, Smith M, Wiggins J, Barnes PJ. Release and activity of matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 by alveolar macrophages from patients with chronic obstructive pulmonary disease. *Am J Respir Cell Mol Biol* 2002;26:602–609.
30. Lim S, Roche N, Oliver BG, Mattos W, Barnes PJ, Fan CK. Balance of matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 from alveolar macrophages in cigarette smokers, regulation by interleukin-10. *Am J Respir Crit Care Med* 2000;162:1355–1360.
31. Capelli A, Di Stefano A, Gnemmi I, Balbo P, Cerutti CG, Balbi B, Lusuardi M, Donner CF. Increased MCP-1 and MIP-1 β in bronchoalveolar lavage fluid of chronic bronchitis. *Eur Respir J* 1999;14:160–165.
32. Traves SL, Smith SJ, Barnes PJ, Donnelly LE. Increased migration of monocytes from COPD patients towards GRO- α is not mediated by an increase in CXCR2 receptor expression [abstract]. *Am J Respir Crit Care Med* 2003;165(Suppl):A824.
33. Soler P, Moreau A, Basset F, Hance AJ. Cigarette smoking-induced changes in the number and differentiated state of pulmonary dendritic cells/Langerhans cells. *Am Rev Respir Dis* 1989;139:1112–1117.
34. Takizawa H, Tanaka M, Takami K, Ohtoshi T, Ito K, Satoh M, Okada Y, Yamasawa F, Nakahara K, Umeda LE. Increased expression of transforming growth factor- β_1 in small airway epithelium from tobacco smokers and patients with chronic obstructive pulmonary disease (COPD). *Am J Respir Crit Care Med* 2001;163:1476–1483.
35. Caramori G, Romagnoli M, Casolari P, Bellettato C, Casoni G, Boschetto P, Chung KF, Barnes PJ, Adcock IM, Ciaccia A, et al. Nuclear localisation of p65 in sputum macrophages but not in sputum neutrophils during COPD exacerbations. *Thorax* 2003;58:348–351.
36. Sassetta M, Di Stefano A, Turato G, Facchini FM, Corbino L, Mapp CE, Maestrelli P, Ciaccia A, Fabbri LM. CD8⁺ T-lymphocytes in peripheral airways of smokers with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 1998;157:822–826.
37. Finkelstein R, Fraser RS, Ghezzi H, Cosio MG. Alveolar inflammation and its relation to emphysema in smokers. *Am J Respir Crit Care Med* 1995;152:1666–1672.
38. Sassetta M, Baraldo S, Corbino L, Turato G, Braccioni F, Rea F, Cavallero G, Tropeano G, Mapp CE, Maestrelli P, et al. CD8⁺ cells in the lungs of smokers with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 1999;160:711–717.
39. Harel S, Janoff A, Yu SY, Hurewitz A, Bergofsky EH. Desmosine radioimmunoassay for measuring elastin degradation *in vitro*. *Am Rev Respir Dis* 1980;122:769–773.
40. Gottlieb DJ, Stone PJ, Sparrow D, Gale ME, Weiss ST, Snider GL, O'Connor GT. Urinary desmosine excretion in smokers with and without rapid decline of lung function: the Normative Aging Study. *Am J Respir Crit Care Med* 1996;154:1290–1295.
41. Gadek J, Fells GA, Crystal RG. Cigarette smoking induces functional antiprotease deficiency in the lower respiratory tract of humans. *Science* 1979;206:1315–1316.
42. Abboud RT, Fera T, Richter A, Tabona MZ, Johal S. Acute effect of smoking on the functional activity of α_1 -protease inhibitor in bronchoalveolar lavage fluid. *Am Rev Respir Dis* 1985;131:1187–1189.
43. Burnett D, Chamba A, Hill SL, Stockley RA. Neutrophils from subjects with chronic obstructive lung disease show enhanced chemotaxis and extracellular proteolysis. *Lancet* 1987;ii:1043–1046.
44. Goldstein RA, Starcher BC. Urinary excretion of elastin peptides containing desmosine after intratracheal injection of elastase in hamsters. *J Clin Invest* 1978;61:1286–1290.
45. Kuhn C, Yu SH, Chraplyvy M, Linder HE, Senior RM. The induction of emphysema with elastase. II. Changes in connective tissue. *Lab Invest* 1976;34:372–380.
46. Fukuda Y, Masuda Y, Ishizaki M, Masugi Y, Ferrans VJ. Morphogenesis of abnormal elastic fibers in lungs of patients with paracinar and centriacinar emphysema. *Hum Pathol* 1989;20:652–659.
47. Wang Z, Zheng T, Zhu Z, Homer RJ, Riese RJ, Chapman HA Jr, Shapiro SD, Elias JA. Interferon γ induction of pulmonary emphysema in the adult murine lung. *J Exp Med* 2000;192:1587–1600.
48. Zheng T, Zhu Z, Wang Z, Homer RJ, Ma B, Riese RJ Jr, Chapman HA Jr, Shapiro SD, Elias JA. Inducible targeting of IL-13 to the adult lung causes matrix metalloproteinase- and cathepsin-dependent emphysema. *J Clin Invest* 2000;106:1081–1093.
49. Takeyabu K, Betsuyaku T, Nishimura M, Yoshioka A, Tanino M, Miyamoto K, Kawakami Y. Cysteine proteinases and cystatin C in bronchoalveolar lavage fluid from subjects with subclinical emphysema. *Eur Respir J* 1998;12:1033–1039.
50. Russell RE, Thorley A, Culpitt SV, Dodd S, Donnelly LE, Demattos C, Fitzgerald M, Barnes PJ. Alveolar macrophage-mediated elastolysis: roles of matrix metalloproteinases, cysteine, and serine proteases. *Am J Physiol Lung Cell Mol Physiol* 2002;283:L867–L873.
51. Atkinson JJ, Senior RM. Matrix metalloproteinase-9 in lung remodeling. *Am J Respir Cell Mol Biol* 2003;28:12–24.
52. Culpitt SV, Maziak W, Loukidis S, Nightingale JA, Matthews JL, Barnes PJ. Effect of high dose inhaled steroid on cells, cytokines, and proteases in induced sputum in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 1999;160:1635–1639.
53. Finlay GA, O'Driscoll LR, Russell KJ, D'Arcy EM, Masterson JB, Fitzgerald MX, O'Connor CM. Matrix metalloproteinase expression

- and production by alveolar macrophages in emphysema. *Am J Respir Crit Care Med* 1997;156:240–247.
54. Betsuyaku T, Nishimura M, Takeyabu K, Tanino M, Venge P, Xu S, Kawakami Y. Neutrophil granule proteins in bronchoalveolar lavage fluid from subjects with subclinical emphysema. *Am J Respir Crit Care Med* 1999;159:1985–1991.
 55. Ohnishi K, Takagi M, Kurokawa Y, Satomi S, Kontinen YT. Matrix metalloproteinase-mediated extracellular matrix protein degradation in human pulmonary emphysema. *Lab Invest* 1998;78:1077–1087.
 56. Imai K, Dalal SS, Chen ES, Downey R, Schulman LL, Ginsburg M, D'Armiento J. Human collagenase (matrix metalloproteinase-1) expression in the lungs of patients with emphysema. *Am J Respir Crit Care Med* 2001;163:786–791.
 57. Morris DG, Huang X, Kaminski N, Wang Y, Shapiro SD, Dolganov G, Glick A, Sheppard D. Loss of integrin α_5 -mediated TGF- β activation causes MMP12-dependent emphysema. *Nature* 2003;422:169–173.
 58. Lanone S, Zheng T, Zhu Z, Liu W, Lee CG, Ma B, Chen Q, Homer RJ, Wang J, Rabach LA, et al. Overlapping and enzyme-specific contributions of matrix metalloproteinases-9 and -12 in IL-13-induced inflammation and remodeling. *J Clin Invest* 2002;110:463–474.
 59. MacNee W. Oxidative stress and lung inflammation in airways disease. *Eur J Pharmacol* 2001;429:195–207.
 60. Pryor WA, Stone K. Oxidants in cigarette smoke: radicals, hydrogen peroxides, peroxyhydrate, and peroxyhydrate. *Ann N Y Acad Sci* 1993; 686:12–28.
 61. Schaberg T, Haller H, Rau M, Kaiser D, Fassbender M, Lode H. Superoxide anion release induced by platelet-activating factor is increased in human alveolar macrophages from smokers. *Eur Respir J* 1992;5: 387–393.
 62. Cantin A, Crystal RG. Oxidants, antioxidants and the pathogenesis of emphysema. *Eur J Respir Dis* 1985;66:7–17.
 63. Laurent P, Janoff A, Kagan HM. Cigarette smoke blocks cross-linking of elastin *in vitro*. *Am Rev Respir Dis* 1983;127:189–192.
 64. Morrison D, Rahman I, Lannan S, MacNee W. Epithelial permeability, inflammation and oxidant stress in the air spaces of smokers. *Am J Respir Crit Care Med* 1999;159:473–479.
 65. Lannan S, Donaldson K, Brown D, MacNee W. Effects of cigarette smoke and its condensates on alveolar cell injury *in vitro*. *Am J Physiol* 1994;266:L92–L100.
 66. Li XY, Donaldson K, Rahman I, MacNee W. An investigation of the role of glutathione in the increased epithelial permeability induced by cigarette smoke *in vivo* and *in vitro*. *Am J Respir Crit Care Med* 1994;149:1518–1525.
 67. Rahman I, Morrison D, Donaldson K, MacNee W. Systemic oxidative stress in asthma, COPD, and smokers. *Am J Respir Crit Care Med* 1996;154:1055–1060.
 68. Morrow JD, Frei B, Longmire AW, Gaziano JM, Lynch SM, Shyr Y, Strauss WE, Oates JA, Roberts LJ II. Increase in circulating products of lipid peroxidation (F_2 -isoprostanes) in smokers. *N Engl J Med* 1995; 332:1198–1203.
 69. Paredi P, Kharitonov SA, Barnes PJ. Analysis of expired air for oxidation products. *Am J Respir Crit Care Med* 2002;166:S31–S37.
 70. Dekhuijzen PN, Aben KK, Dekker I, Aarts LP, Wielders PL, van Herwaarden CL, Bast A. Increased exhalation of hydrogen peroxide in patients with stable and unstable chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 1996;154:813–816.
 71. Montuschi P, Collins JV, Ciabattini G, Lazzeri N, Corradi M, Kharitonov SA, Barnes PJ. Exhaled 8-isoprostane as an *in vivo* biomarker of lung oxidative stress in patients with COPD and healthy smokers. *Am J Respir Crit Care Med* 2000;162:1175–1177.
 72. Rahman I, van Schadewijk AA, Crowther AJ, Hiemstra PS, Stolk J, MacNee W, De Boer WI. 4-Hydroxy-2-nonenal, a specific lipid peroxidation product, is elevated in lungs of patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2002;166:490–495.
 73. Wesseliuss LS, Nelson ME, Skikne BS. Increased release of ferritin and iron by iron-loaded alveolar macrophages in cigarette smokers. *Am J Respir Crit Care Med* 1994;150:690–695.
 74. Cantin AM, Fells GA, Hubbard RC, Crystal RG. Antioxidant macromolecules in the epithelial lining fluid of the normal human lower respiratory tract. *J Clin Invest* 1990;86:962–971.
 75. Linden M, Hakansson L, Ohlsson K, Sjodin K, Tegner H, Tunek A, Venge P. Glutathione in bronchoalveolar lavage fluid from smokers is related to humoral markers of inflammatory cell activity. *Inflammation* 1989;13:651–658.
 76. Rahman I, Li XY, Donaldson K, Harrison DJ, MacNee W. Glutathione homeostasis in alveolar epithelial cells *in vitro* and lung *in vivo* under oxidative stress. *Am J Physiol* 1995;269:L285–L292.
 77. Pacht ER, Kaseki H, Mohammed JR, Cornwell DG, Davis WR. Deficiency of vitamin E in the alveolar fluid of cigarette smokers. influence on alveolar macrophage cytotoxicity. *J Clin Invest* 1988;77:789–796.
 78. Bui MH, Sauty A, Collet F, Leuenberger P. Dietary vitamin C intake and concentrations in the body fluids and cells of male smokers and nonsmokers. *J Nutr* 1992;122:312–336.
 79. Gilks CB, Price K, Wright JL, Churg A. Antioxidant gene expression in rat lung after exposure to cigarette smoke. *Am J Pathol* 1998;152: 269–278.
 80. Rahman I, MacNee W. Regulation of redox glutathione levels and gene transcription in lung inflammation: therapeutic approaches. *Free Radic Biol Med* 2000;28:1405–1420.
 81. Keicho N, Elliott WM, Hogg JC, Hayashi S. Adenovirus E1A upregulates interleukin-8 expression induced by endotoxin in pulmonary epithelial cells. *Am J Physiol* 1997;272:L1046–L1052.
 82. Elliott WM, Hayashi S, Hogg JC. Immunodetection of adenoviral E1A proteins in human lung-tissue. *Am J Respir Cell Mol Biol* 1995;12:642–648.
 83. Vitalis TZ, Karn I, Croome A, Behzad H, Hayashi S, Hogg JC. The effect of latent adenovirus 5 infection on cigarette smoke-induced lung inflammation. *Eur Respir J* 1998;11:664–669.
 84. Gilmour PS, Rahman I, Hayashi S, Hogg JC, Donaldson K, MacNee W. Adenoviral E1A primes alveolar epithelial cells to PM₁₀-induced transcription of interleukin-8. *Am J Physiol Lung Cell Mol Physiol* 2001;281:L598–L606.
 85. Higashimoto Y, Elliott WM, Behzad AR, Sedgwick EG, Takei T, Hogg JC, Hayashi S. Inflammatory mediator mRNA expression by adenovirus E1A-transfected bronchial epithelial cells. *Am J Respir Crit Care Med* 2002;166:200–207.
 86. Ito K, Lim S, Caramori G, Chung KF, Barnes PJ, Adcock IM. Cigarette smoking reduces histone deacetylase 1 expression, enhances cytokine expression, and inhibits glucocorticoids actions in alveolar macrophages. *FASEB J* 2001;15:1110–1112.
 87. Marwick JA, Kirkham PA, Stevenson CS, Danahay H, Giddings J, Butler K, Donaldson K, MacNee W, Rahman I. Cigarette smoke alters chromatin remodelling and induces pro-inflammatory genes in rat lungs. *Am J Respir Cell Mol Biol* 2004;31:633–642.
 88. Szulakowski P, Drost E, Jimenez LS, Wickenden JA, Crowther AJ, Donaldson K, MacNee W. Role of histone acetylation in transcription regulation and promotion of inflammatory process in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2004;169:A275.
 89. To Y, Elliott WM, Ito M, Hayashi S, Adcock IM, Hogg JC, Barnes PJ, Ito K. Total histone deacetylase activity decreases with an increasing clinical stage of COPD [abstract]. *Am J Respir Crit Care Med* 2004; 169:A276.
 90. Golpon HA, Coldren CD, Zamora MR, Cosgrove GP, Moore MD, Tudor RM, Geraci MW, Voelkel NF. Emphysema lung tissue gene expression profiling. *Am J Respir Cell Mol Biol* 2004;31:595–600.
 91. Spira A, Beane J, Pinto-Plata V, Kadar A, Liu G, Shah V, Celli B, Brody JS. Gene expression profiling of human lung tissue from smokers with severe emphysema. *Am J Respir Cell Mol Biol* 2004;31:601–610.
 92. Tudor RM, Petrache I, Elias JA, Voelkel NF, Henson PM. Apoptosis and emphysema: the missing link. *Am J Respir Cell Mol Biol* 2003;28: 551–554.
 93. Kasahara Y, Tudor RM, Cool CD, Lynch DA, Flores SC, Voelkel NF. Endothelial cell death and decreased expression of vascular endothelial growth factor and vascular endothelial growth factor receptor 2 in emphysema. *Am J Respir Crit Care Med* 2001;163:737–744.
 94. Kasahara Y, Tudor RM, Taraseviciene-Stewart L, Le Cras TD, Abman SH, Hirth P, Waltenberger J, Voelkel NF. Inhibition of vascular endothelial growth factor receptors causes lung cell apoptosis and emphysema. *J Clin Invest* 2000;106:1311–1319.
 95. Kanazawa H, Asai K, Hirata K, Yoshikawa J. Possible effects of vascular endothelial growth factor in the pathogenesis of chronic obstructive pulmonary disease. *Am J Med* 2003;114:354–358.
 96. Aoshiba K, Yokohori N, Nagai A. Alveolar wall apoptosis causes lung destruction and emphysematous changes. *Am J Respir Cell Mol Biol* 2003;28:555–562.
 97. Tudor RM, Zhen L, Cho CY, Taraseviciene-Stewart L, Kasahara Y, Salvemini D, Voelkel NF, Flores SC. Oxidative stress and apoptosis interact and cause emphysema due to vascular endothelial growth factor receptor blockade. *Am J Respir Cell Mol Biol* 2003;29:88–97.
 98. Imai K, Mercer BA, Schulman LL, Sonett JR, Armiento JMD. Correlation of lung surface area to apoptosis and proliferation in human emphysema. *Eur Respir J* 2005;25:250–258.
 99. Saetta M, Di Stefano A, Maestrelli P, Ferrarasso A, Drigo R, Potena

- A, Ciaccia A, Fabbri LM. Activated T-lymphocytes and macrophages in bronchial mucosa of subjects with chronic bronchitis. *Am Rev Respir Dis* 1993;147:301–306.
100. O'Shaughnessy TC, Ansari TW, Barnes NC, Jeffery PK. Inflammation in bronchial biopsies of subjects with chronic bronchitis: inverse relationship of CD8⁺ T lymphocytes with FEV₁. *Am J Respir Crit Care Med* 1997;155:852–857.
101. Ollerenshaw SL, Woolcock AJ. Characteristics of the inflammation in biopsies from large airways of subjects with asthma and subjects with chronic airflow limitation. *Am Rev Respir Dis* 1992;145:922–927.
102. Saetta M, Turato G, Facchini FM, Corbino L, Lucchini RE, Casoni G, Maestrelli P, Mapp CE, Ciaccia A, Fabbri LM. Inflammatory cells in the bronchial glands of smokers with chronic bronchitis. *Am J Respir Crit Care Med* 1997;156:1633–1639.
103. Zhu J, Majumdar S, Qiu Y, Ansari T, Oliva A, Kips JC, Pauwels RA, De Rose V, Jeffery PK. Interleukin-4 and interleukin-5 gene expression and inflammation in the mucus-secreting glands and sub-epithelial tissue of smokers with chronic bronchitis: lack of relationship with CD8⁺ cells. *Am J Respir Crit Care Med* 2001;164:2220–2228.
104. Bosken CH, Hards J, Gatter K, Hogg JC. Characterization of the inflammatory reaction in the peripheral airways of cigarette smokers using immunocytochemistry. *Am Rev Respir Dis* 1992;145:911–917.
105. Lams BE, Sousa AR, Rees PJ, Lee TH. Immunopathology of the small-airway submucosa in smokers with and without chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 1998;158:1518–1523.
106. Grashoff WF, Sont JK, Sterk PJ, Hiemstra PS, de Boer WI, Stolk J, Han J, van Krieken JM. Chronic obstructive pulmonary disease: role of bronchiolar mast cells and macrophages. *Am J Pathol* 1997;151:1785–1790.
107. De Boer WI, van Schadewijk A, Sont JK, Sharma HS, Stolk J, Hiemstra PS, van Krieken JH. Transforming growth factor β_1 and recruitment of macrophages and mast cells in airways in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 1998;158:1951–1957.